Salmonella enterica Prevalence in The Ohio State University Veterinary Medical Center Environment

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Abstract

Salmonella is an important enteric pathogen of humans and animals. In a veterinary teaching hospital, the health risk associated with direct *Salmonella* exposure threatens the safety of patients, staff, and students. The objectives of this study are to measure the frequency of *Salmonella* in The Ohio State University Veterinary Medical Center (OSU-VMC) hospital environment, and determine if there are resident *Salmonella* strains which are maintained. Samples were aseptically collected with an electrostatic cloth from twenty combined floor drains from the equine (n = 10) and food animal (n = 10) areas of the OSU-VMC between February 16, 2015, and November 3, 2015. The samples were added to buffered peptone water, transferred to Rappaport-Vassiliadis broth, and inoculated onto XLT-4 and MacConkey agar. To determine if bacterial growth was *Salmonella,* a polyvalent antisera test was performed and the isolates were inoculated onto TSI slants. Pulsed-field gel electrophoresis (PFGE) was used to determine bacterial relatedness using banding patterns of recovered environmental *Salmonella* isolates and eight clinical isolates recovered from food animal and equine patients between February 6, 2015, and May 20, 2016. A total of 23 *Salmonella* isolates were recovered from 360 (6.4%) environmental samples with prevalence ranging from 0 to 40% on 18 individual sampling dates. A total of 8.9% of food animal drain samples and 3.9% of equine drain samples were positive for *Salmonella*. The PFGE indicates that eight unique environmental *Salmonella* strains were recovered. None of the eight clinical isolates had similar PFGE banding patterns to the environmental isolates. No single *Salmonella* strains persisted within the OSU-VMC environment for an extended period of time. The presence of the same environmental *Salmonella* clones recovered from both equine drains and food animal drains on the same date of sampling suggests that *Salmonella* are being transferred between the two areas, and possibly to the entire hospital.

Introduction

Salmonella are a gram-negative, rod-shaped bacteria that can cause serious gastroenteritis in humans [Salmonella Infection (2014), Salmonella (2015)]. *Salmonella* inhabits the gastrointestinal tract of infected animals and humans, and is transmitted through direct contact without proper hygiene or through other modes of fecal-oral transmission. Nearly 42,000 human *Salmonella* cases are confirmed annually leading to an average of over 23,000 hospitalizations and nearly 500 deaths (Scallan et al. 2011).

Animal environments can become contaminated with *Salmonella* [(Hoelzer et al. 2011), (Hendriksen SWM et al. 2004)]., thus, making food animal populations an important reservoir for human infection (Hendriksen SWM et al. 2004). Consumption of raw milk from infected cattle is known to facilitate the spread of *Salmonella* to humans (Hoelzer et al. 2011). In addition, direct animal contact with infected cattle have contributed to the dissemination of *Salmonella* (Hoelzer et al. 2011). Multiple *Salmonella* outbreaks have been linked to environmental contamination in areas where cattle are present without appropriate hand hygiene (Hoelzer et al. 2011). *Salmonella* can contaminate healthcare environments, including veterinary hospitals; for extended periods of time (Dunowska et al. 2007). The health effects of *Salmonella* infection can range from mild gastrointestinal upset to death in both animals and humans in serious cases [Hoelzer et al. (2011), *Salmonella* (2001)].

A *Salmonella* outbreak at the Colorado State University Veterinary Teaching Hospital in 1996 led to the closure of the large animal ward in order to prevent the dispersal of *Salmonella* to other animals and staff (Dunowska et al. 2007). Another *Salmonella* outbreak at the University of Pennsylvania Veterinary Teaching Hospital over the course of ten months led to nosocomial infections and the closure of the hospital to prevent further dissemination of *Salmonella* to patients and staff (Dallap Schaer, B.L. et al. 2010). An outbreak at the Michigan State University large animal clinic in 1996 led to the closure of the entire hospital to prevent further nosocomial infection of multi-drug resistant *Salmonella* spp. amongst the equine patients (Schott II et al. 2001).

Salmonella environmental surveillance has been previously conducted at the OSU-VMC in 2007 and 2009. Seven clonal strains were recovered in 2007 which were resistant to 3 drug classes (streptomycin, sulfamethozxole, and tetracycline), respectively. Strains were unique to

the food animal section and the equine section, but no strains were recovered from both (Pandya et al. 2007) Twelve *Salmonella* isolates were recovered in 2009, all of which were resistant only to sulfisoxazole. Of the ten isolates that were serotyped nine were *S.* Muenster and one was 18:autoagglutinator (Roberts et al. 2009). Both studies determined *Salmonella* spp. were more readily recovered from floor drains in the food animal section of the OSU-VMC. The objectives of this study were to determine the prevalence of *Salmonella* contamination in the OSU-VMC environment, and determine if there are nosocomial strains that show antibiotic resistance in order to analyze the possible risks associated with the students and staff.

Materials and Methods

Sampling

Each week from February 16, 2015, to April 20, 2015, and again from August 20, 2015, to November 3, 2015, ten floor drains each from the equine and food animal areas of the OSU-VMC were sampled for a total of twenty samples per visit. The samples were aseptically collected by swabbing the cover grate of each drain with an electro-static cloth and place into a sterile whirlpak bag for transport to the laboratory.

Bacterial Culture

Immediately following sample collection, 90 mL of Buffered Peptone Water (BPW) was added to each sample. The sample was then incubated at 37°C for 18-24 hours. 100 μL of the BPW/sample broth was transferred into a 10 mL Rappaport-Vassiliadis (RV) broth and incubated at 42°C for 24-48 hours. A sterile swab was used to inoculate the RV onto Xylose-Lysine-Tergitol 4 (XLT-4) agar and streaked for isolation. The XLT-4 agar were incubated for 24 hours at 37°C. A single phenotypic black colony was inoculated and streaked for isolation onto MacConkey agar. The MacConkey agar were incubated at 37°C for 18-24 hours and examined for phenotypic lactose negative growth. A single isolated colony selected from the MacConkey agar was used to perform a polyvalent antisera test, and inoculated to a Triple Sugar Iron (TSI) slant and then incubated for 24 hours at 37°C. The TSI slant was analyzed for a black precipitate and no pH color indicator change in the TSI agar. An isolated colony from the MacConkey agar was then inoculated to nutrient agar and incubated at 37°C for 18-24 hours. The nutrient agar was checked for growth and then stored at 4°C for future reference. The

National Veterinary Services Laboratory (NVSL) performed the serotyping of the isolates using standard agglutination procedures.

Minimum Inhibitory Concentration (MIC)

To determine MICs, the isolates were inoculated onto Luria-Bertani (LB) agar and incubated at 37°C for 18-24 hours. Approximately 0.5 mm^2 of bacteria from the LB agar was added to 2 mL double distilled water in a Falcon tube. A Genesys 20 spectrophotometer set to 625 nm wavelength was utilized to maintain a 0.5 McFarland standard. The spectrophotometer was utilized to obtain between 0.08 and 0.1 bacterial concentration within the Falcon tube. Then 10 uL of the diluted bacteria was added to 5 mL of Muller-Hinton (MH) broth, and 50 uL of the MH broth was transferred into each well of the MIC panel (Sensititre, Remel) using a 300 uL multichannel pipette. After inoculation, the panels were covered with film and incubated at 35°C for 18-24 hours.

Pulsed-Field Gel Electrophoresis (PFGE)

To examine the genetic similarity of *Salmonella* isolates, pulsed-field gel electrophoresis (PFGE) genotyping (CHEF-DRIII; Bio-Rad Laboratories, Hercules, CA) was performed on total genomic DNA. Agarose plugs prepared with the *Salmonella* isolates were digested using XbaI (Promega, Madison, WI) following previously reported protocols (Ribot et al., 2006). After electrophoresis, banding patterns were compared and levels of similarity assigned using generally accepted criteria (Tenover et al., 1995). *Salmonella* isolates were compiled into pulsotypic groups by using the Dice coefficient similarity index and the unweighted pair-group method with arithmetic averages (UPGMA) with clustering settings of 1.00% optimization and 1.00% band position tolerance via Bionumerics software (Applied Maths, Kortrijik, Belgium).

Results

A total of 23 *Salmonella* isolates were recovered from the 360 samples (6.4%). More *Salmonella* isolates were recovered during the Spring sampling (13.1%) compared to Autumn sampling (1.0%). Sixteen of the 23 recovered isolates were from the Food Animal area of the OSU-VMC, for an 8.9% prevalence; while the remaining 7 isolates were discovered in the Equine area attributing to a 3.9% prevalence. A dendrogram was constructed from the PFGE

results to determine clonality amongst the environmental and clinical isolates which is displayed in Figure 1. Eight unique clonal strains were identified based on PFGE (Figure 1) and a representative isolate from each was submitted for serotyping. Three were identified as *S.* Montevideo, one *S.* Agbeni, one *S.* Muenster, one *S.* Meleagridis, one *S.* Anatum, and one *S.* Cerro. Clonal strains were recovered from multiple drains in both equine and food animal service areas on the same sampling date, and the same clonal strain was recovered over two consecutive sampling dates. Figure 2 summarizes the minimum inhibitory concentrations of the *Salmonella* isolates. The MIC data shows that 9 isolates are pan-susceptible to all the drug classes, while 13 isolates are resistant to one drug class. One isolate is resistant to 4 drug classes, those being sulfisoxazole, chloramphenicol, streptomycin, and tetracycline. More isolates resistant to one or more drug classes were recovered from the equine service area. No clonality was present between the clinical isolates and the environmental isolates, nor was any clonality present between the clinical isolates.

An odds ratio was constructed and the odds of a drain being *Salmonella* positive is 2.41 times higher ($P = 0.06$) in the food animal service area compared to the equine service area.

Discussion

These results suggest that *Salmonella* can remain within the OSU-VMC hospital environment over the course of at least a week and potentially longer. It is likely that the environmental *Salmonella* were introduced into the OSU-VMC hospital environment by infected large animal patients. The environmental *Salmonella* may have been dispersed throughout the food animal and equine service areas by regular veterinary students and staff movement between the two areas. With this in mind, it is possible that the environmental *Salmonella* was introduced into other areas of the hospital given the close proximity of the companion animal service areas. It is possible that patients may shed *Salmonella* into the hospital environment without showing signs of clinical salmonellosis. Of the eight patient diagnostic isolates, only two were patients in the OSU-VMC at the time of our environmental sampling; therefore, the remaining six patients could have been shedding *Salmonella* into the environment without infecting other patients.

The higher recovery of *Salmonella* from the food animal ward was expected due to the high reported frequency of *Salmonella* infection and shedding in dairy cattle.

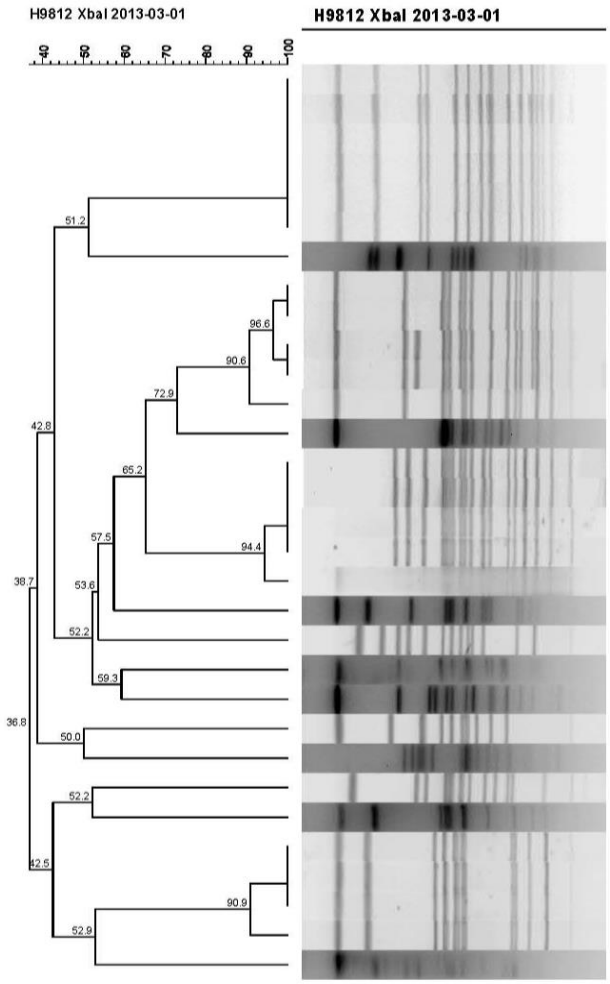
The cleaning and disinfection protocols at the OSU-VMC appear to be effective at removing environmental contamination of *Salmonella* and preventing resident strains. However, the environmental strains we observed can be re-introduced into the hospital environment due to patients remaining within the OSU-VMC for an extended period of time during treatment. *Salmonella* surveillance is warranted for all food animal and equine patients showing signs of diarrhea to reduce the number of animals shedding *Salmonella* within the OSU-VMC hospital environment. The presence of *Salmonella* in The Ohio State University Veterinary Medical Center puts all students and staff in the hospital at risk for contracting this important zoonotic disease. The potential health risk associated with *Salmonella* cannot be overlooked as students are conducting their clinical rotations in the same environment as the infected equine and cattle. The safety of the other patients in the hospital environment is also of concern due to the possible nosocomial transmission.

Table 1. Recovery of *Salmonella* from drains located in the food animal and equine sections of the Ohio State University Veterinary Medical Center by sections, date of recovery, and drain location.

Isolate	Positive Samples	Total Samples	Prevalence
Hospital environment	23	360	6.4
Food Animal service	16	180	8.9
Equine service	7	180	3.9
Spring sampling	21	160	13.1
Fall sampling	2	200	1.0
Dates:			
2-16-2015	0	20	0
2-23-205	6	20	30
3-9-2015	4	20	20
3-16-2015	0	20	0
3-30-2015	0	20	0
4-6-2015	8	20	40
4-13-2015	0	20	0
4-20-2015	2	20	10
8-24-2015	0	20	0
8-31-2015	2	20	0
9-7-2015	0	20	0
9-14-2015	1	20	5
9-21-2015	0	20	0
9-28-2015	0	20	0
10-12-2015	0	20	0
10-20-2015	0	20	0
10-27-2015	0	20	0
11-3-2015	0	20	0
Drain:			
Equine 1	0	18	0
Equine 2	1	18	5.6
Equine 3	1	18	5.6
Equine 4	0	18	0
Equine 5	1	18	5.6
Equine 6	1	18	5.6
Equine 7	1	18	5.6
Equine 8	2	18	11.1
Equine 9	0	18	0
Equine 10	0	18	0
Food Animal 1	1	18	5.6

Food Animal 2	0	18	0
Food Animal 3	2	18	11.1
Food Animal 4	2	18	11.1
Food Animal 5	1	18	5.6
Food Animal 6	3	18	16.7
Food Animal 7	2	18	11.1
Food Animal 8	3	18	16.7
Food Animal 9	2	18	11.1
Food Animal 10	0	18	0

Figure 1. Dendrographic analysis of *Salmonella* isolates recovered from floor drains and patient diagnostic isolates from the Ohio State University Veterinary Medical Center.



Isolate		Date	Resistance	Serotype
Equine	8	Feb 23	Sul	
Food animal	3	Feb 23	Sul	
Equine	5	Feb 23	Sul	Anatum
Equine	6	Feb 23	Pan-susceptible	
Equine	2	Feb 23	Sul	
Equine	3	Feb 23	Sul	
Clinical	4	Feb 6	---	
Food animal	4	Mar 9	Pan-susceptible	
Food animal	9	Mar 9	Sul	Montevideo
Food animal	6	Mar 9	Sul	
Food animal	8	Mar 9	Sul	
Food animal	7	Aug 31	Sul	Agbeni
Clinical	7	Sept 24	---	
Food animal	3	Apr 6	Pan-susceptible	
Food animal	1	Apr 6	Pan-susceptible	
Food animal	4	Apr 6	Pan-susceptible	
Food animal	6	Apr 6	Pan-susceptible	Montevideo
Food animal	5	Apr 6	Pan-susceptible	
Clinical	6	May 20, 2016	---	
Equine	7	Aug 31	Chl, Str, Sul, Tet	Montevideo
Clinical	2	May 12, 2016	---	
Clinical	8	Mar 27, 2016	---	
Equine	8	Apr 20	Sul	Muenster
Clinical	5	Dec 2	---	Dublin
Food animal	6	Sep 14	Pan-susceptible	Meleagridis
Clinical	3	Nov 6	---	
Food animal	7	Apr 6	Sul	
Food animal	8	Apr 6	Sul	Cerro
Food animal	9	Apr 6	Sul	
Food animal	8	Apr 20	Pan-susceptible	
Clinical	1	July 18	---	

Sul represents Sulfisoxazole. Str represents Streptomycin. Chl represents Chloramphenicol. Tet represents Tetracycline. Pan-susceptible represents no resistance to any drug class included in the NARMS panel.

Figure 2. Minimum inhibitory concentrations of 23 environmental *Salmonella* isolates recovered from floor drains in the food animal and equine sections of the Ohio State University Veterinary Medical Center.

ug/ml	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Amoxicillin/Clavulanic Acid							9	14								
Ampicillin							11	12								
Azithromycin								3	20							
Ceftoxitin							1	13	8	1						
Ceftiofur							21	2								
Ceftriaxone					22	1										
Chloramphenicol									10	12			1			
Ciprofloxacin	20	3														
Gentamicin					1	13	6	3								
Naladixic Acid								8	15							
Streptomycin										6	10	6		1		
Sulfisoxazole													4	4	1	14
Tetracycline									21	1			1			
Trimethoprim/Sulfamethoxazole				22	1											

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